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BRIEF COMMUNICATION

Transient Suppression of a Secondary Humoral Response in Rats Is Evoked by Lithium–Pilocarpine-Induced Limbic Seizures

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FALTER, H., M. A. PERSINGER AND R. CHRÉTIEN. *Transient suppression of a secondary humoral response in rats is evoked by lithium–pilocarpine-induced limbic seizures.* PHARMACOL BIOCHEM BEHAV **43**(1) 315–317, 1992.— Several experiments were designed to evaluate a secondary humoral response following limbic seizures. After baseline antigen binding capacity (ABC) had been determined for the primary response, a second subcutaneous injection of the antigen (human serum albumin) was accompanied by an injection of either lithium (3 mEq/kg)–pilocarpine (30 mg/kg) or one of two comparator treatments: metrazol (30 mg/kg) or cyclophosphamide (50 mg/kg); other rats served as drug controls. Only the groups that received the lithium–pilocarpine (status epilepticus) or cyclophosphamide (no seizure) displayed significant immunosuppression after 5 but not 10 days. The results support the hypothesis that seizure activity within the amygdaloid–hippocampal complex modulates immunocompetence through corticotropin mechanisms.

Limbic seizures Lithium Metrazol ACTH Corticosteroid Cyclophosphamide Rats Pilocarpine Amygdala Entorhinal cortices

THE hippocampus and amygdala exert strong modulatory control over the hypothalamus; it affects the responsivity of both the humoral and cellular components of the immune system. Facilitation or suppression of immunologic responses are phasic phenomena that reflect periods of "overshoot" and "undershoot" to sudden changes in homeostasis. Circulating levels of the immunomodulator neuropeptide corticotropin (ACTH) are known to be influenced by duration of and severity of limbic seizures (3). We decided to determine if a single systemic injection of lithium (Li) and pilocarpine, sufficient to evoke limbic status epilepticus (4,5), could evoke both elevated ACTH levels and transient immunosuppression of a secondary humoral response. Histomorphology strongly suggests that the initial stages of these seizures originate within the amygdala, pyriform cortices, and entorhinal–hippocampal region (5).

METHOD

In four experiments, a total of 125 100- to 120-day-old Wistar male rats served as subjects. They had been housed three per cage within a standard temperature- and light-controlled (12 L:12 D cycle, light onset 0800 h) environment for at least 2 weeks. Rat chow and water were available ad lib. For Experiment 1, each rat was injected SC in the right flank with 0.1 ml 10 μg/ml human serum albumin (HSA) in normal saline; for Experiment 2, each rat received the HSA in a comparable volume of complete Freund's adjuvant. Twenty days later, each rat was placed in a restraint cage and blood was quickly removed (mean = 4.2 min, SD = 1.8 min) from the tail vein for the baseline measure of antibody binding capacity (ABC); an identical amount of HSA (booster) with no adjuvant was then injected. Consequent blood samples were also taken 5 and 10 days after the booster.

Determination of antigen binding capacity of the rat sera utilized the Farr (2) method and has been described in detail elsewhere (1). Our a priori definition of a responder to HSA was a level of at least 0.11 μg/ml ABC on the day of the baseline sample. Only rats that displayed this criteria were used in subsequent analyses; there were 22% nonresponders in Experiment 1 and 5% in Experiment 2.

All treatments were administered after the booster. In Ex-

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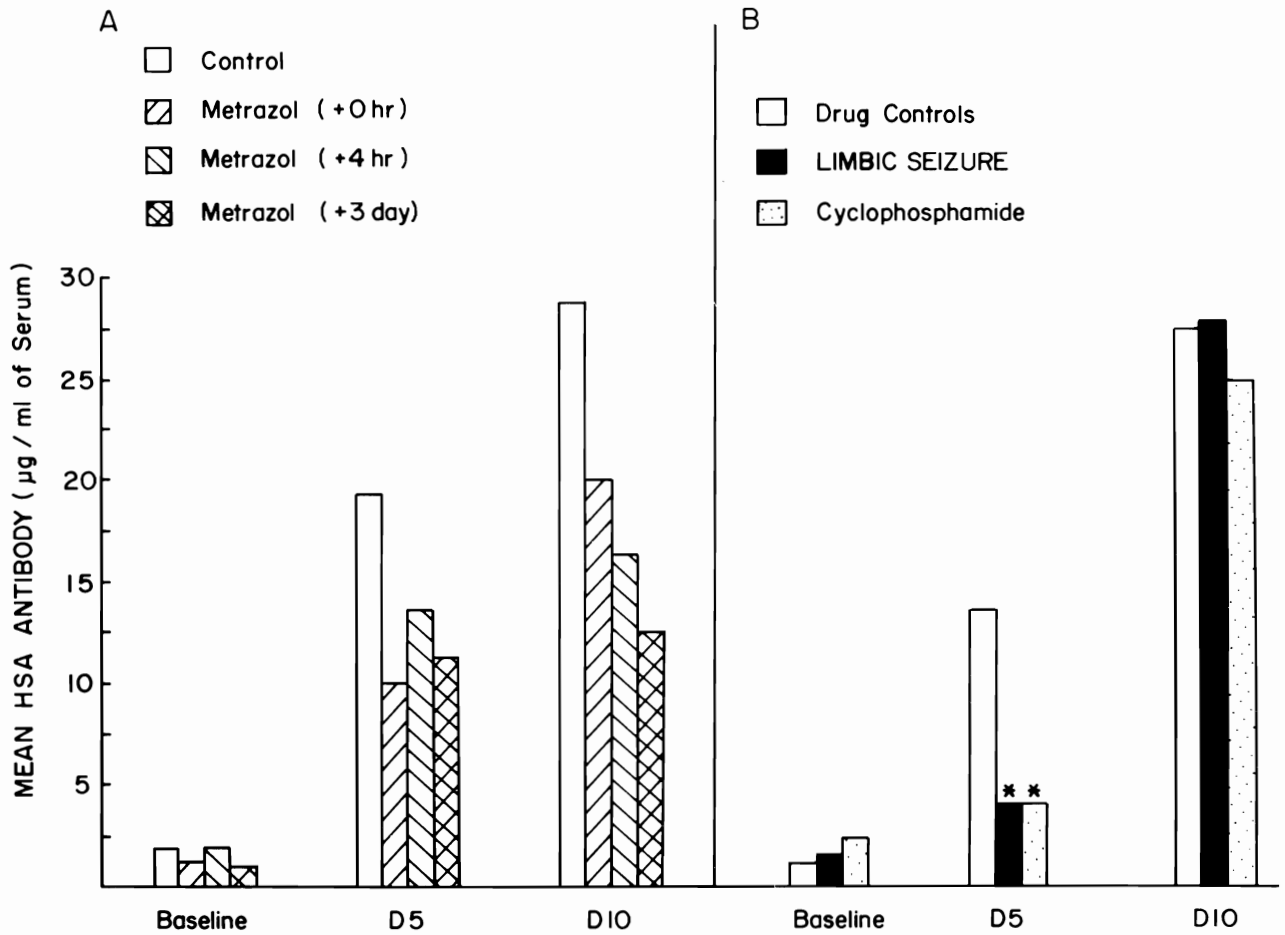

FIG. 1. (A) Mean antigen binding capacity (ABC) in microgram per ml of serum for the baseline and for days 5 and 10 after rats received the HSA booster and received seizure-inducing metrazol immediately, 4 h later, or 3 days later; controls were not injected. (B) ABC measures for rats: a) in which limbic seizures (status) were evoked by a single injection of lithium and pilocarpine (sc; 4 h between drugs), b) that received either lithium or pilocarpine (but not both; no seizures), and c) that received 50 mg/kg cyclophosphamide IP; all treatments given within a few hours of baseline/booster.

TABLE 1

MEANS AND SDS FOR THE LOG (BASE 10) RATIOS OF HSA ANTIBODY (μG/ML SERUM) LEVELS FOR DAY 5/BASELINE AND DAY 10/BASELINE AFTER VARIOUS TREATMENTS

Treatment		Day 5/Baseline		Day 10/Baseline	
Label	*n*	Mean	SD	Mean	SD
Experiment 1A					
Controls	10	0.94	0.77	1.11	0.76
Metrazol (0 h)	7	0.75	0.54	0.95	0.63
Metrazol (4 h)	6	1.00	0.32	1.06	0.42
Metrazol (3 days)	10	0.95	0.24	1.03	0.31
Experiment 1B					
Drug controls	11	1.03*	0.33	1.40*	0.32
Limbic seizure (lithium/pilocarpine 4 h)	9	0.34†	0.47	1.11	0.64
Cyclophosphamide	9	0.07‡	0.11	0.90†	0.22
Experiment 2					
Lithium/pilocarpine (4 h)	11	0.47*	0.41	1.04*	0.60
Lithium/pilocarpine (24 h)	11	1.14†	0.36	1.90†	0.64

* vs. †: $p < 0.05$; † vs. ‡: $p < 0.05$; * vs. ‡: $p < 0.01$.

periment 1A, rats were randomly assigned (9–12/group) to groups that received 30 mg/kg metrazol IP either immediately, 4 h after, or 3 d after the booster; a fourth group was not injected. In Experiment 1B, rats were injected SC with Li (3 mEq/kg) and then 4 h later with 30 mg/kg of pilocarpine (n = 14), identical dosages and volumes of saline, Li or pilocarpine (n = 12), or cyclophosphamide (50 mg/kg; n = 9). In Experiment 2A, 12 rats received Li 24 h while another 12 received Li 4 h before injection of pilocarpine and the baseline blood extraction. All rats received 25 mg/kg acepromazine, which facilitates recovery from status epilepticus (5). Body weight was measured every 2 days.

In Experiment 2B, ACTH was determined from trunk blood samples (four/group) taken either 30 min, 6 h, or 24 h after induction of the Li/pilocarpine seizures; controls that had been moved into the experimental room but not treated and nondisturbed rats were also employed. ACTH levels were determined by a kit purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). Corticosterone was measured for baseline and day 5 blood serum samples for another 24 Li/pilocarpine and control rats according to the radioimmunoassay marketed by ICN Biomedicals.

RESULTS

The absolute ABCs for the secondary response are presented in Fig. 1 while the ratios of the ABCs on posttreatment days 5 and 10 relative to the baseline (primary) are shown in Table 1. Multivariate analyses of variance (MANOVAs) revealed the expected secondary response over days, $F(1, 29) = 15.95$, $p < 0.001$, but no treatment or day × treatment differences for the metrazol groups. However, MANOVA demonstrated significant, $F(2, 26) = 11.68$, $p < 0.001$, group differences between the control, limbic seizure, and cyclophosphamide groups, $F(2, 26) = 11.68$, $p < 0.001$, and a significant treatment × days interaction, $F(2, 26) = 5.31$, $p = 0.01$. A posteriori analyses indicated that the interaction was due to the relatively greater suppression of the secondary response on day 5 for the limbic seizure and cyclophosphamide groups compared to the controls; one way analysis of variance (ANOVA) for the ABC/baseline ratios verified this effect, $F(2, 26) = 21.49$, $p < 0.001$, for day 5 only (Table 1).

Primary (baseline) and secondary (booster) responses, as defined by ABC, for rats in Experiment 2 were about three to five times greater than in Experiment 1. The means and SDs for the posttreatment/baseline ratios of ABC responses on days 5 and 10 for the two Li/pilocarpine temporal intervals (4, 24 h) are shown in Table 1. [For comparison to Experiment 1, the HSA antibody levels (μg/ml serum) for the 4- and 24-h groups were 4.4 (4.0) and 23.3 (27.3) on day 5 and 18.7 (15.2) and 181.2 (130.1) on day 10, respectively].

Rats that received the seizure-inducing combination at the interval (4 h) that evoked immunosuppression on posttreatment day 5 in Experiment 1 also displayed significantly, $F(1, 20) = 16.29$, $p < 0.001$, greater immunosuppression of the same magnitude relative to rats that received pilocarpine 24 h after Li; the group difference explained 45% of the variance in ABC. However, by day 10, rats in which seizures had been evoked by the 24-h Li–pilocarpine treatment displayed greater immunofacilitation, $F(1, 20) = 10.74$, $p = 0.003$.

There were no statistically significant differences in the usual loss in body weight (5) that follows the seizures for the 4- and 24-h interval groups ($p > 0.05$); the percentage weight loss for both groups combined for days 5 and 10 were 85 (5)% and 89 (9)%, respectively. There was no significant correlation between any of the ABC measures and relative loss of body weight. Although there were no significant correlations between corticosterone levels (grand mean = 93 ng/ml, SD = 78 ng/ml) and either absolute body weight (grand mean = 430 g, SD = 30 g) or ABC levels during baseline, a negative correlation (-0.48, $p = 0.01$) existed between percentage body weight relative to baseline and corticosterone levels on day 5.

The means and SDs (in parentheses) for ACTH (pg/ml serum) were: (a) control, 59(13); (b) room disruption controls, 182(50); (c) limbic seizure + 30 min, 500+(0); (d) limbic seizure + 6 h, 145(39); and (e) limbic seizure + 24 h, 162(25). Kruskal–Wallis demonstrated significant ($\chi^2 = 13.80$, $p < 0.01$) group differences. ANOVA and a posteriori tests with the four groups that displayed variance indicated the following group differences: 2 = 5 = 4 > 1.

DISCUSSION

We have completed many unpublished experiments involving behavioral (restraint, handling) manipulations that did not affect either the primary or secondary humoral response. The results of this study indicate that limbic seizures, sufficient to produce significant necrosis in the brain, evoke transient suppressions and facilitation in humoral responses. These seizures are associated with transient extreme increases in ACTH.

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